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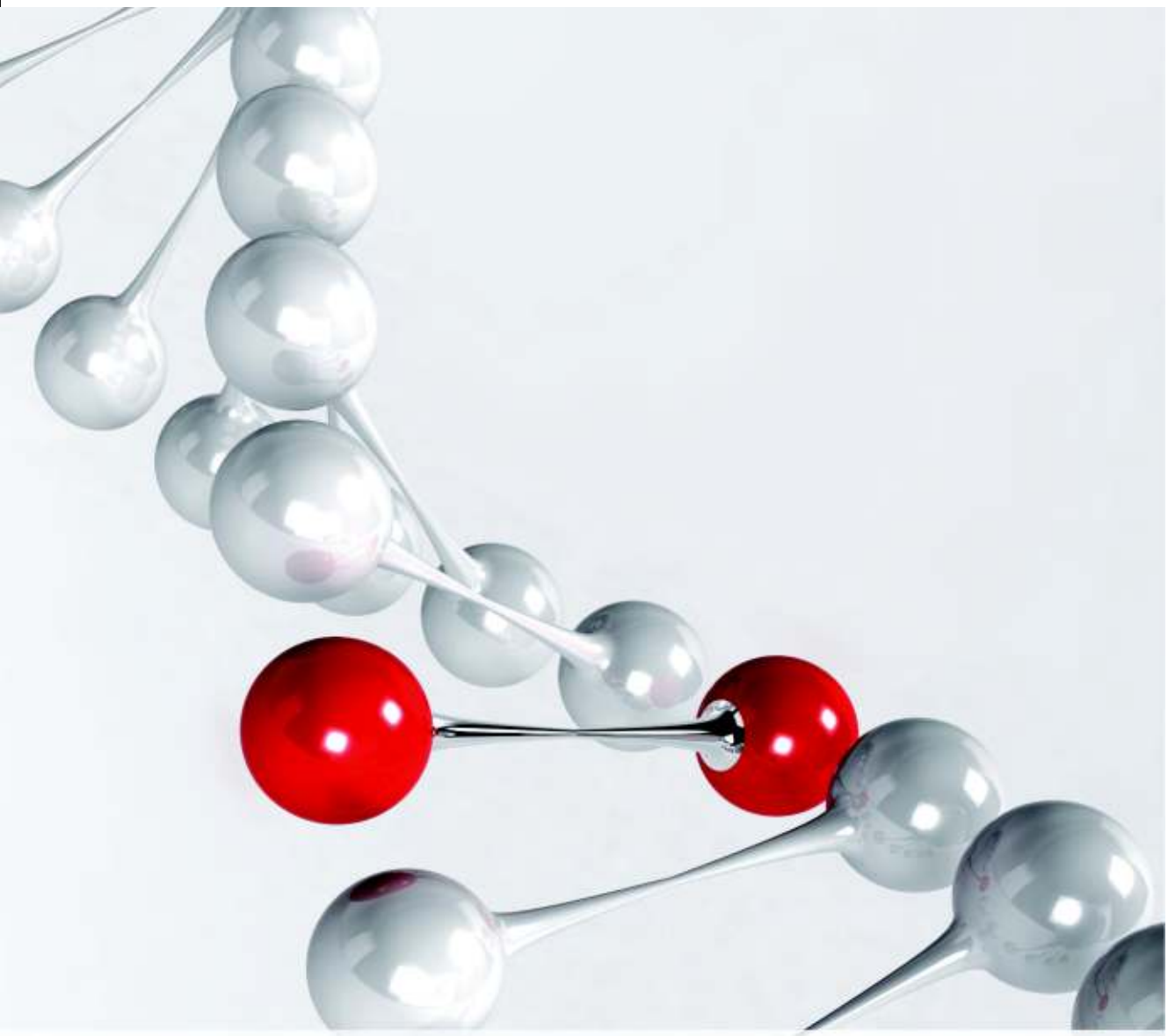

User manual
Invisorb® Universal Bacteria HTS 96 Kit/ C

for purification of bacterial or parasite DNA from different kinds of starting materials in a 96-well format using a centrifuge

CE
IVD

REF 7033300x00

STRATEC Molecular GmbH, D-13125 Berlin

Invisorb® Universal Bacteria HTS 96 Kit

The **Invisorb® Universal Bacteria HTS 96 Kit** is the ideal tool in convenient 96 well format for isolation and purification of bacterial DNA from different clinical specimen, e.g. whole blood, swabs, biopsy material and from cell free body fluids, e.g. urine, serum, plasma and synovial fluids.

Invisorb® Universal Bacteria HTS 96 Kit suits to manual and automated*) use. Ultra pure genomic DNA gets purified for enhanced performance in sensitive applications. Additionally, yields of the ready to use DNA are reproducible. Large sample numbers in this 96 well format are handled time-saving. The user can be flexible in his choices in the range of starting material. No cross-contamination will occur.

The kit is neither validated for the isolation of human or bacterial genomic DNA from faecal samples nor for viral nucleic acids or RNA isolation.

Trademarks: Invisorb®, VACUUBRAND are registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

Invisorb® is a registered trademark of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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*) for automated use, please contact in Germany 030 9489 2901/ 10 or your local distributor.

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Kit contents of the Invisorb® Universal Bacteria HTS 96 Kit/ C

Store all kit components at room temperature.
Diluted Proteinase K at –20°C! Lyophilized Lysozyme should be stored at –20°C. Dissolved Lysozyme must be stored in aliquots at –20°C.

	2 x 96 DNA extractions	4 x 96 DNA extractions	24 x 96 DNA extractions
Catalogue No.	7033300200	7033300300	7033300400
Lysozyme Buffer	25 ml	45 ml	250 ml
Lysozyme	250 mg	450 mg	2,5 g
Proteinase K	3 x 1.5 ml	8.5 ml	50 ml
Lysis Buffer HL	50 ml	90 ml	500 ml
Binding Buffer HL	2 x 8 ml final volume 2 x 32 ml	30 ml final volume 120 ml	130 ml final volume 520 ml
Wash Buffer I	80 ml final volume 160 ml	2 x 80 ml final volume 2 x 160 ml	2 x 450 ml final volume 2 x 900 ml
Wash Buffer II	2 x 45 ml final volume 2 x 150 ml	3 x 60 ml final volume 3 x 200 ml	4 x 270 ml final volume 4 x 900 ml
Elution Buffer	30 ml	60 ml	250 ml
DNA-Binding Plate B	2	4	6 x 4
2.0 ml Collection Plate	4	2 x 4	12 x 4
Elution Plate L	2	4	24
Plate Lid	2	4	24
Sealing Foils	4	8	48
Manual	1	1	1
Initial Steps	add 24 ml 99.7% Isopropanol to each **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. add 80 ml EtOH to the bottle Wash Buffer I add 105 ml EtOH to each bottle Wash Buffer II add 1.5 ml ddH_2O to each tube Proteinase K and store at –20°C add provided amount of Lysozyme to the bottle Lysozyme Buffer and mix it thoroughly. Aliquot and store at -20°C	add 90 ml 99.7% Isopropanol to the **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. add 80 ml EtOH to each bottle Wash Buffer I add 140 ml EtOH to each bottle Wash Buffer II add 8.5 ml ddH_2O to the tube Proteinase K and store at –20°C add provided amount of Lysozyme to the bottle Lysozyme Buffer and mix it thoroughly. Aliquot and store at -20°C	add 390 ml 99.7% Isopropanol to the **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. add 450 ml EtOH to each bottle Wash Buffer I add 630 ml EtOH to each bottle Wash Buffer II add 50 ml ddH_2O to the tube Proteinase K and store at –20°C add provided amount of Lysozyme to the bottle Lysozyme Buffer and mix it thoroughly. Aliquot and store at -20°C

Symbols

Manufacturer

Lot number

Catalogue number

Date of manufacture

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit contents of the **Invisorb® Universal Bacteria HTS 96 Kit**, except **MAP Solution A** and **Lysozyme** should be stored at room temperature and are stable for at least 12 months under these conditions.

Lysozyme: Lyophilized **Lysozyme** should be stored at –20°C. Dissolved **Lysozyme** must be stored in aliquots at –20°C. Avoid repeated freezing and thawing.

Proteinase K: Dissolved **Proteinase K** must be stored at -20 °C.

Wash Buffer I and II: Wash Buffer charged with ethanol should be stored at room temperature and should be appropriate sealed. If there are any precipitates within the provided solutions solve these precipitates by careful warming up to room temperature (up to 30 °C). **Room temperature is defined as range from 15 – 30°C.**

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **Invisorb® Universal Bacteria HTS 96 Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.
STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.
In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **Invisorb® Universal Bacteria HTS 96 Kit** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.
If you have any questions or problems regarding any aspects of **Invisorb® Universal Bacteria HTS 96 Kit** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany +49-(0)30-9489-2901/ 2910
from abroad +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **Invisorb® Universal Bacteria HTS 96 Kit** has been designed to extract highly pure DNA from up to 100 µl of fresh or frozen blood, plasma, serum, cell free body fluids of human origin like urine, as well as rinsed liquid from swabs or cell pellets in a 96 well format using a centrifuge. Fresh or frozen blood, plasma or serum from common blood collection systems can be used with EDTA or citrate, *but not with heparin*.

Final extracted bacterial DNA is of high quality and suitable for a wide variety of downstream applications. For reproducible and high yields appropriate sample storage is essential. Yields may vary from sample to sample depending on factors such as the health of the donor, patient medication, or sample storage conditions. The purified DNA is of high quality and free of proteins, nucleases and other impurities and is ready to use for different downstream applications like PCR, quantitative PCR, real time PCR or other routine diagnostic methods.

Any diagnostic results generated using the sample preparation procedure in conjunction with any downstream diagnostic assays should be interpreted with regard to other clinical o laboratory finding

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONAL USERS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

Product use limitation

The kit is neither validated for the isolation of human or bacterial genomic DNA from faecal samples nor for viral nucleic acids or RNA isolation.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications. STRATEC Molecular Products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The Product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **Invisorb® Universal Bacteria HTS 96 Kit** procedure for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **Invisorb® Universal Bacteria HTS 96 Kit** procedure to which they apply are listed below as follows.

Lysis Buffer HL

danger
H302-315-319 P280-305-351-338

Proteinase K

danger
H315-319-334-335 P280-305-351-338-310-405

Wash Buffer I

warning
H302-312-332-412 EUH032 P273

H302:	Harmful if swallowed.
H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.
P273:	Avoid release to the environment.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
in USA : 1 – 800 – 535 – 5053

Product characteristic of the Invisorb® Universal Bacteria HTS 96 Kit

Starting material	Yield	Time for preparation	Ratio
up to 100 µl whole blood, up 5 - 10 mg tissue up to 100 µl cell free body fluids (serum, plasma, synovial liquid, urine) swabs	depending on the amount and kind of starting material	about 80 min	A_{260} : A_{280} 1.7 – 1.9

The **Invisorb® Universal Bacteria HTS Kit** is designed for isolation and purification of bacterial DNA from bacterial pellets or different clinical samples, e.g. up to 100 µl of fresh or frozen blood, plasma, serum, cell free body fluids of human origin like urine, as well as rinsed liquid from swabs or cell pellets on a 96 well format.
The kit composition of the **Invisorb® Universal Bacteria HTS Kit/ C** is designed for use on a centrifuge.

In a manual step all samples are normalized to a sample volume of 100 µl before using. Small samples should be adjusted to 100 µl with 1x PBS or water before starting the protocol. The kit is designed for simultaneous processing of multiple samples.

The samples are pretreated with **Lysozyme** at 37°C to break the bacterial cell wall and lysed in an optimized lysis buffer. Proteins are degraded during the lysis with **Proteinase K** at 65°C. The lysis efficiency is improved by shaking the samples in the 96 well plate during the lysis. The DNA binds to a filter membrane, followed by washing steps and the final elution. The procedure requires minimal interaction by the user, allowing safe handling of potentially infectious samples. The procedures are designed to avoid sample-to-sample cross-contamination.

The **Invisorb® Universal Bacteria HTS 96 Kit** yield highest quality of DNA, so the obtained DNA can be used directly in:

- PCR
- Real time PCR
- RFLP analysis
- Restriction Enzyme Digestion

Proven **Invisorb® 96 technology** provides uniform DNA recovery and purity across the 96-well plate with no cross-contamination between samples.

Protocol Validation

Verification Testing

Bacterial DNA extraction protocol was functionally tested on a centrifuge using **Invisorb® Universal Bacteria HTS 96 Kit/ C** provided reagents and consumables. Typical results for the extraction of bacterial DNA from buffer, plasma and blood are shown below. Actual results will vary depending upon sample age, quality, type, and species of subject.

Samples

For testing frozen cell pellets of the gram-positive bacterium *Bacillus subtilis* were used in different dilutions of cells and in different matrices. The cells were grown in an over night culture and cell pellets from 1ml of this culture is stored at –20°C until use. For all experiment always a fresh pellet was taken from –20°C and the cells were discarded after the use for one day. The detection was done by an in-house *Bacillus subtilis* real-time PCR based detection assay. All real-time PCR's were performed on a Corbett Rotor-Gene 3000.

PCR Inhibitor and Cross Contamination Test

To maximize the detection of any potential contamination event, positive and no sample controls were arranged in alternating wells (in a “checkerboard” pattern ***Fig. 1***). Out of those samples ***Fig. 2*** shows a real-time PCR run of the extracted DNA. PCR were done with an in-house SYBR Green based real–time PCR assay on a Corbett Rotor-Gene 3000 machine.

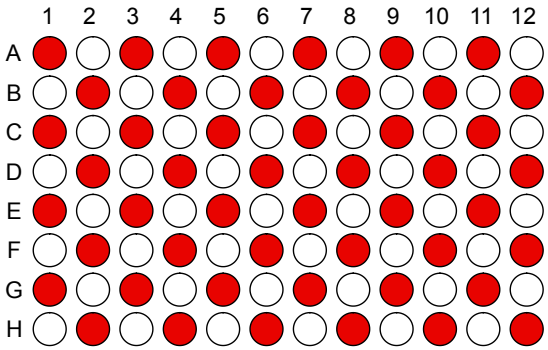


Fig. 1 **‘Checkerboard Pattern’** utilized for the cross contamination analysis test. Samples (red) and no sample controls (white) arranged in alternating wells.

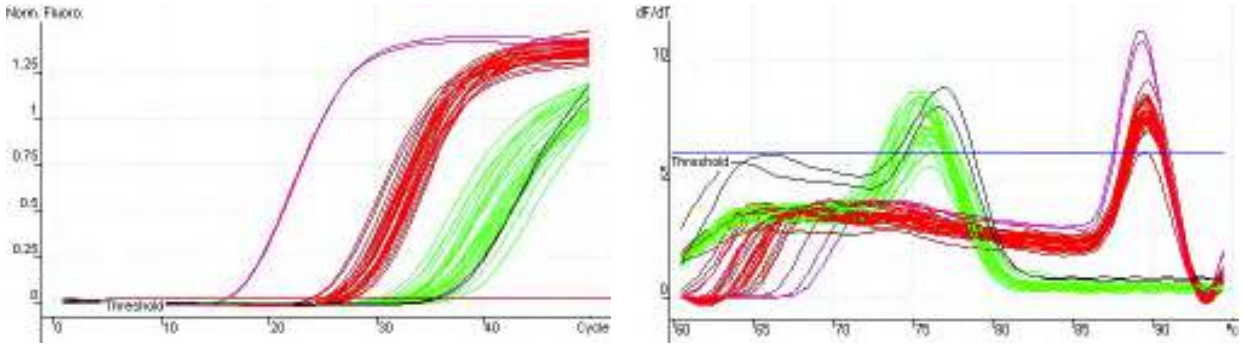


Fig. 2 Real-time PCR results from positive samples (red) and no sample controls (green) arranged in a Checkerboard. NTC (no template control, black) and PTC (positive template control, pink) are also shown.

Tab. 1 To show the high reproducibility of the Ct mean value and the standard deviation for the samples in ***Fig. 2*** the results are listed below.

Colour	Name	Rep. Ct	Rep. Ct Std. Dev.
	pos.	26.46	0.95
	neg.	33.95	1.75
	PTC	16.41	0.03
	NTC	36.11	0.39

Reproducibility

To show the reproducibility of the **Invisorb® Universal Bacteria HTS 96 Kit** different bacterial DNA extractions were done with dilution series. Typical results are shown below.

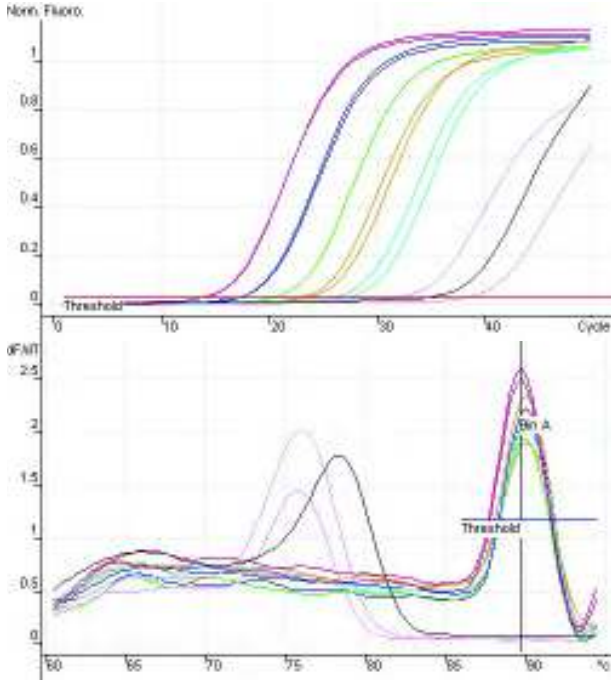


Fig. 3 On the left side real-time PCR result of an extracted DNA from a dilution series of *B. subtilis* cells in TE buffer. On the right side the melting curve for the resulting PCR products.

Tab. 2 Ct values and standard deviations for the real-time PCR shown above in figure 4. Colors belongs to the curves in figure 3.

Colour	Name	mean Ct	mean Ct Std. Dev.
	10e-3	17.63	0.09
	10e-4	20.92	
	10e-5	23.63	0.36
	10e-6	27.03	1.08
	10e-7	35.58	4.57
	PTC	14.39	0.14
	NTC	35.39	

Influence of different matrices

To shown the usability of different clinical relevant matrices the same amount of *B. subtilis* cell were spiked to 100µL of whole blood, plasma, urine and TE buffer and the DNA extracted following the kit. Resulting DNA were analyzed by real-time PCR. Results are shown below.

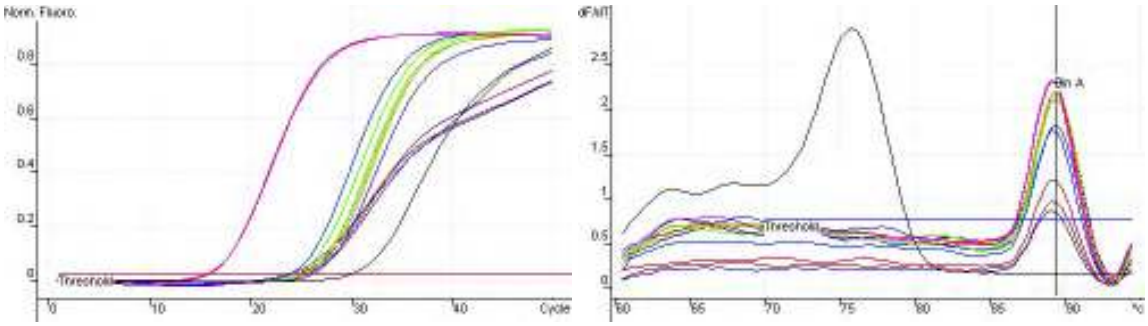


Fig. 4 Real-time PCR results from DNA extracted from the same amount of *B. subtilis* cell from different matrices. On the left amplification data on the right melting curves for the resulting PCR products.

Tab. 3 Ct values for the extraction of the same amount of B. subtlis from different matrices.

Colour	Name	Rep. Ct	Rep. Ct Std. Dev.
	TE buffer 10e-5	**25.46**	1.57
	blood 100 µL 10e-5	**25.30**	1.19
	blood 50 µL 10e-5	**25.60**	0.44
	plasma 100 µL 10e-5	**25.48**	0.61
	urine 100 µL 10e-5	**25.98**	0.28
	PTC	16.21	0.07
	NTC	30.50	

Tab. 4 Ct value and standard deviation over all sample shown in table 3.

Rep. Ct	Rep. Ct Std. Dev.
25.56	0.75

Investigation of the recovery of DNA from *Staphylococcus aureus*

To test the kit with other bacteria than *B. subtilis* a test with two different inactivated *Staphylococcus aureus* were done. The *Staphylococcus aureus* were from a bouillon inactivated with 1 volume of 96% ethanol. The concentrations of cells were unknown.
For the experiment 50 µl and 5 µl were used and filled up to 100 µl with TE buffer. The extraction was done following the Manual. The detection was done with the "SureClin MRSA PLUS V" Kit from Congen Biotechnologie GmbH. Results are shown below. From this kit only the results for the *S. aureus* detection are shown.

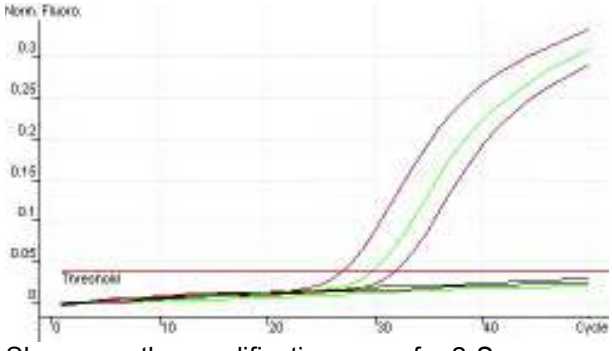


Fig. 5 Shown are the amplification curve for 2 *S. aureus* positive samples (red and green) and the NTC black (no template control).

Tab. 5 Ct values for the detection of *S. aureus* (FAM) and the internal amplification control (JOE).

Colour	Name	Genotype	Ct FAM	Ct JOE
	1 aureus 50	S. aureus positive	27.33	34.32
	2 mrsa 50	S. aureus positive	30.09	34.34
	1 aureus 5	S. aureus positive	32.19	34.47
	2 mrsa 5	S. aureus negative		34.38
	PTC	S. aureus positive	33.71	37.22
	PTC	S. aureus positive	33.02	35.26
	NTC	S. aureus negative		30.60
	NTC	S. aureus negative		30.62

Results

Reproducibility of PCR values showed no evidence of PCR inhibitors in any of the extracted bacterial DNA samples. Also dilutions and comparison between different extraction and matrices showed high reproducibility. The usability of more clinical relevant bacteria was demonstrated with inactivated *S. aureus*, but the results are not directly comparable with real clinical samples.

Principle and procedure

The **Invisorb® Universal Bacteria HTS 96 Kit** procedure comprises following steps:

1. sample pre-treatment and normalization of all samples to a volume of 100 µl
2. lysis of bacterial cells
3. binding the DNA to the membrane
4. washing the membrane and elimination of ethanol
5. elution of total DNA (genomic, bacterial, mitochondrial)

The samples will be lysed in an optimized buffer and enzyme mixture. The lysates are transferred to the subsequent purification procedure based on silica membranes. The DNA binds to membrane, followed by washing steps and the final elution. The purified high quality DNA is ready to use for subsequent downstream applications e.g. for PCR amplification, quantitative PCR, real time PCR or other routine diagnostic methods

This instruction contains 6 protocols.

Sampling and storage of starting material

Blood/ buffy coat: Blood samples and buffy coat can be stored at room temperature (18-25°C) up to 6 hours, or at 2-8°C up to 24 hours. For long term storage, we recommend freezing samples at –20°C or –80°C. Multiple thawing and freezing before isolating the DNA should be avoided. If cryoprecipitates (formed during thawing of frozen samples) are visible, avoid aspirating them during aspiration of the sample, they could clog the **Filterplate**. The amount of purified DNA in the **Invisorb® Universal Bacteria HTS 96 Kit/ C** procedure from max. 100 µl whole blood or 25 µl buffy coat depends on the white blood cell content of each blood sample. Various different primary tubes and anticoagulants (except heparin) can be used to collect blood samples for the **Invisorb® procedure.**

Biopsy material: Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor quality starting material also leads to reduced length and yield of purified DNA. The amount of purified DNA in the **Invisorb® Universal Bacteria HTS 96 Kit/ C** procedure from max. 10 mg biopsy sample depends on the nature of starting material.

Body fluids (plasma, serum, synovial fluids, urine): Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. The amount of purified DNA in the **Invisorb® Universal Bacteria HTS 96 Kit/ C** procedure from max. 100 µl body fluids (e.g. amniotic fluid, synovial fluid) depends on the nature and amount of cells.

Swabs*:* The protocol works with fresh prepared swabs as well as with dried swabs. Please note, that stored and dried swab sample often characterized by isolation of apoptotic DNA (visible on agarose gel as typical apoptotic DNA banding pattern).You can use also up to 100µl rinsed liquid from swab. The protocol has not been validated for isolation of DNA from swabs which are stored under a storage buffer.

Bacterial cultures**:** Bacterial cultures grow in the presence of a selective agent such as an antibiotic. The yield and quality of DNA may depend on factors such host strain, inoculation, antibiotic, and type of culture medium. The bacteria will be pelleted after cultivation. Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.

Urine: The bacteria must be pelleted and the supernatant completely removed (urea contaminations can inhibit PCR reactions). Best results are obtained with fresh peletted material or bacteria pellets that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. The amount of purified DNA from max. 15-50 ml urine, depends from the included bacteria titre.

Pathogens in food material *(Listeria ssp.):*For the detection of bacteria (Listeria) in foods they must be enriched and cultivated following the EU regulations and § 35 of the food law. An aliquot of the culture will only be used and the bacteria will be pelleted after cultivation. Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.

Procedure

Normalization
Each sample is mixed with PBS or ddH_2O and filled up to a final volume of 100 µl.

Sample preparation
Bacteria must be cultivated under special conditions and an aliquot of the bacteria suspension will be used to get a bacteria pellet by centrifugation at high speed for 5 min. The supernatant will be removed.

Lysis
Lysis is performed in several steps – at first in the presence of **Lysozyme** to break the cell wall of the bacteria. Then samples are lysed under denaturing conditions at different elevated temperatures and continuously shaking using a **Lysis Buffer** and **Proteinase K** to digest the proteins. DNases are inactivated. The bacterial DNA is secured. Unlysed sample parts should be removed before the binding step.

Binding genomic DNA
By adding **Binding Buffers HL** to the lysate, optimal binding conditions are achieved. Each lysate is then applied to a well of the **DNA Binding Plate B** and genomic DNA is adsorbed onto the membrane.

Removing residual contaminants
Contaminants are efficiently washed away using **Wash Buffers** while the bacterial, genomic DNA remains bound to the membrane.

Elution of pure genomic DNA
Genomic DNA is eluted from the Invisorb® 96 Filter plate using 100 µl Elution Buffer. The eluted DNA is ready for use in different downstream applications. Long-term studies on the stability of eluates are still in progress.

Important notes

Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Important indications

1. Optimal disruption of tissue is important for obtaining maximum yield and purity of genomic DNA. For high sample throughput it is recommended to use a Mixer Mill with a 96 insert.

2. The elution can be done by using lower amount of Elution Buffer (min. 75 µl). This may result in a higher DNA-concentration. Eluting twice with each with 75 µl Elution Buffer is recommended and produces lightly higher yield.

3. Eluting with pre-warmed Elution Buffer up to 80°C increases yield.

4. It is recommended to centrifuge the isolated DNA for 1 min at maximum speed before starting any application.

5. All centrifugations are hold at room temperature.

6. To avoid cross-contaminations plates have to be sealed with Sealing Foils.

7. It is recommended to use multi channel pipettes, esp. electric multi channel pipettes with a capacity of 1 ml.

8. Bacteria must be cultivated under special conditions and an aliquot of the bacteria suspension is used to win a bacteria pellet by centrifugation at high speed for 5 min. The supernatant is removed.

Sample preparation

For further information contact STRATEC Molecular.
Bacteria must be cultivated under special conditions and an aliquot of the bacteria suspension is used to get a bacteria pellet by centrifugation at high speed for 5 min. The supernatant is removed.

General considerations

A summary of sample setup is described in the table below:

Sample and sample size	Condition	Treatment
10 - 100 µl blood	fresh / frozen with anticoagulants	fill up to 100 µl with ddH_2O
10 - 100 µl serum, plasma	fresh / frozen	fill up to 100 µl with ddH_2O
15 - 50 ml urine	fresh, frozen	spin down the sample and resuspend the pellet in 100 µl ddH_2O
5 – 10 mg tissue sample	fresh, frozen	homogenize sample completely by crushing and fill up to 100 µl with ddH_2O or 1x PBS Buffer
5 – 10 mg biopsy	fresh, frozen	
5 – 10 mg frozen section	fresh, frozen	
swabs	fresh / dried	fill up to 100 µl with buffer (0,4% SDS, 50mM Tris-HCl; pH 8.0); or use 120 µl of transport medium or rinse liquid
10 – 100 µl bacterial culture	fresh, frozen	fill up to 100 µl with ddH_2O
cell pellet from 1x 10^9 bacteria	fresh, frozen	resuspend in 100 µl ddH_2O

Table 1

9. Sample treatment before starting (also see table 1)

Blood, buffy coat, saliva, synovial fluid: see table 1
Urine: Spin down up to 50 ml and discard the supernatant, suspend the cells in ddH_2O
Tissue: Homogenize or crush the tissue sample and fill it in a 2 ml 96 Deep Well Plate
Swab: Can be provided in transportation medium – take 120 µl of this media an mix it with 80 µl ddH_2O
An untreated swab (fresh or dried) must be mixed with special buffer (see table) and the supernatant is filled in the well (the yield can be increased if the swab remains in the well during the lysis)

Preparing reagents and buffers

Prior to each isolation
Before starting a run, bring all reagents to room temperature. Where necessary, gently mix and re-dissolve any precipitates by warming to 30°C until dissolved. Swirl gently to avoid foaming.

Lysis Buffer HL and **Elution Buffer** are ready to use**.**

Proteinase K
Add described amount of ddH_2O (see table below) to needed tube or bottle of **Proteinase K**, mix thoroughly and store not needed **Proteinase K** at –20 °C. Dividing the **Proteinase K** into aliquots to avoid repeated freezing and thawing is recommended.

Lysozyme
Lysozyme should be prepared directly before starting the isolation. Solve the lyophilized Lysozyme within the bottle of Lysozyme Buffer. Mix thoroughly until all of the Lysozyme is solved. Store not directly used Lysozyme solution in aliquots at –20°C and avoid repeated freezing and thawing.

Wash Buffer I and **Wash Buffer II**
Before use add the described volume of 96-100% ethanol to the bottle with Wash Buffer I and II as described below. After adding the ethanol mix shortly and keep the bottles always firmly closed!

2 x 96 DNA-extractions:
Add 24 ml 99.7% Isopropanol to each **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 80 ml 96 - 100% ethanol to the bottle **Wash Buffer I** Add 105 ml 96 - 100% ethanol to the bottle **Wash Buffer II** Add 1.5 ml ddH_2O to the **Proteinase K.** Mix thoroughly and store at -20°C Add provided amount of **Lysozyme** to the bottle with the **Lysozyme Buffer** and mix it thoroughly Aliquot and store at -20°C **(100 µl Lysozyme mixture /sample)**
4x 96 DNA-extractions:
Add 90 ml 99.7% Isopropanol to the **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 80 ml 96 - 100% ethanol to the bottle **Wash Buffer I** Add 140 ml 96 - 100% ethanol to the bottle **Wash Buffer II** Add 8.5 ml ddH_2O to the **Proteinase K.** Mix thoroughly and store at -20°C Add provided amount of **Lysozyme** to the bottle with the **Lysozyme Buffer** and mix it thoroughly Aliquot and store at -20°C **(100 µl Lysozyme mixture /sample)**
24x 96 DNA-extractions:
Add 390 ml 99.7% Isopropanol to the **Binding Buffer HL.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 450 ml 96 - 100% ethanol to the bottle **Wash Buffer I** Add 630 ml 96 - 100% ethanol to the bottle **Wash Buffer II** Add 50 ml ddH_2O to the **Proteinase K.** Mix thoroughly and store at -20°C Add provided amount of **Lysozyme** to the bottle with the **Lysozyme Buffer** and mix it thoroughly Aliquot and store at -20°C **(100 µl Lysozyme mixture /sample)**

1. Adjusting the thermomixer , water bath, incubation oven or others to 65°C.

2. To increase the yield: warming up the needed amount of Elution Buffer to 65°C (per sample 1x 100 µl Elution Buffer are needed)

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS) (see our webpage: www.stratec.com).

- Measuring cylinder (250 ml)
- Disposable gloves
- Multichannel pipette with tips
- Reagents reservoirs for multichannel pipettes
- 99.8 % ethanol
- Isopropanol*
- Centrifuge: output ≥ 2.000 x g is necessary **
    - Sigma-Centrifuge 4-15C or centrifuge 4K15C with plate-rotor 2 x 96 or other rotors
    - Eppendorf Centrifuge 5804 / 5804 R / 5810 / 5810 R with Deepwell-Plate-Rotor (A-2-DWP)

*The **Invisorb® Universal Bacteria HTS 96 Kit/ C** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

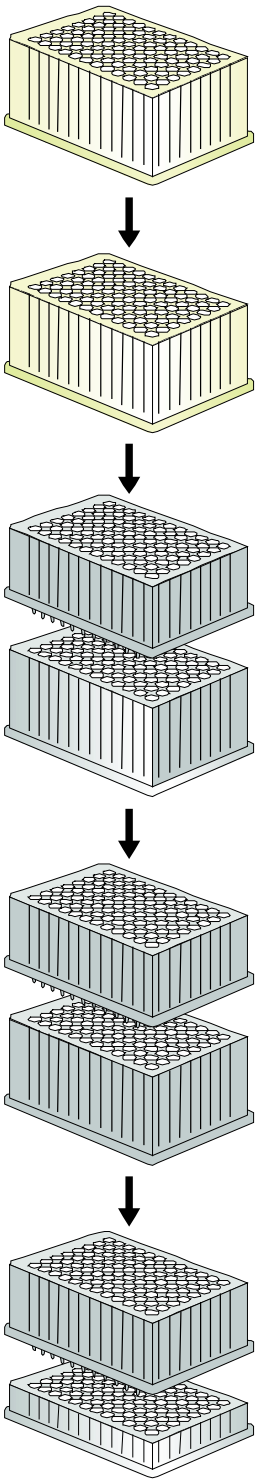
Sigma
2-Propanol
Order no. 59304-1L-F

****Possible supplier for centrifuges:**

KNF Neuberger GmbH
Alter Weg 3
D-79112 Freiburg
Phone: +49 (0) 7664 5909 0
Fax: +49 (0)7664 5909-99
E-Mail: info@knf.de

VACUUBRAND GMBH+CO KG
Alfred-Zippe-Str. 4
D-97877 Wertheim
Phone: +49 (0) 9342 808 0
Fax: +49 (0) 9342 59880
E-Mail: info@vacuubrand.de

Scheme of Invisorb® Universal Bacteria HTS 96 Kit/ C



Please read protocols prior to the start of the preparation

Normalization: each sample smaller than 100 µl must be mixed with ddH_2O, or PBS-buffer, filled up to a final volume of 100 µl and transferred in a **2 ml Collection Plate**

Add 100 µl **diluted Lysozyme to each sample**
Incubate at RT for 10 min
Add 20 µl **Proteinase K** to each sample Mix completely
Incubate at 65 °C for 10 min (800 rpm)
Add 200 µl **Lysis Buffer HL** to each well and mix completely
Incubate at 65°C under continuously shaking for 3 min (800 rpm)

Add 200 µl **Binding Buffer HL** *(follow preparing instructions)* to each well, mix completely

place the DNA Binding Plate B on the top of a 2 ml Collection Plate
transfer lysates completely to the DNA Binding Plate B
incubate at RT for 1 min·
centrifuge at 1.400- 1.700 x g (3.700 – 4.000 rpm) for 6 min at RT or until all lysate was running trough
discard filtrate and place the DNA Binding Plate B back on the top of a 2 ml Collection Plate

add 600 µl **Wash Buffer I** to each well of DNA Binding Plate B
centrifuge at 1.400 - 1.700 x g (3.700 – 4.000 rpm) for 3 min at RT
discard filtrate and place the DNA Binding Plate B back on the top of a 2 ml Collection Plate

add 700 µl Wash Buffer II to each well of DNA Binding Plate B
centrifuge at 1.400 - 1.700 x g (3.700 – 4.000 rpm) for 3 min at RT
discard filtrate and place the DNA Binding Plate B back on the top of a 2 ml Collection Plate

Repeat the Wash Buffer II step

remove all waste from the Collection Plate
centrifuge at maximum speed for 10 min to dry the membrane
discard 2 ml Collection Plate

place the DNA Binding Plate B on the top of a Elution Plate L
add prewarmed 100 µl **Elution Buffer** per cavity
incubate 2 min at RT·
centrifuge at 1.400 - 1.700 x g (3.700 – 4.000 rpm) for 3 min

Protocol 1: Purification of bacterial DNA from clinical samples (serum, plasma, blood and cell free body fluid sample) using a centrifuge

Please read the protocols prior to the start of the preparation and complete preparing steps

Attention: *Please be aware, that you have to prepare the Binding Buffer HL – see page 16*

Sample Preparation

Use up to 100 µl whole blood, plasma or other cell free body fluid. It is recommended for smaller sample volumes than 100 µl to adjust it to 100 µl with 1x PBS or water before starting the purification and isolation procedure.

If sample are very viscous take care not to clog the membrane with this samples. In cases of very viscous samples use smaller sample volumes and adjust to 100 µl with water or 1x PBS.

1. Transfer the adjusted sample (to 100 µl) into one well of the **2.0 ml Collection Plate** and proceed the purification on the **Centrifuge**.

2. Add 100 µl diluted Lysozyme to each well of the **2 ml Collection Plate** and incubate for 10 min at RT.

3. Add 20 µl of prior diluted Proteinase K to each well of the **2 ml Collection Plate**, vortex shortly for stirring (therefore cover wells with a sealing foil) or mix completely by pipetting up and down.
   Incubate the covered plate at 65 °C for about 10 min under continuous shaking (800 rpm).

4. Remove the cover and add 200 µl of Lysis Buffer HL to each well, vortex shortly for stirring (therefore cover wells with a sealing foil) or mix completely by pipetting up and down. Incubate the covered plate at 65 °C for about 3 min under continuous shaking (800 rpm).

Note: *Incubation at RT instead of at 65°C during step 3 and 4 may reduce the lysis efficiency for some bacteria*

5. Add 200 µl **Binding Buffer HL** to each well of the **2 ml Collection Plate** and mix thoroughly by pipetting up and down. **Cover the Collection Plate with the Plate Lid.**
   Place the 2 ml Collection Plate into a centrifuge and spin shortly (centrifuge up to 200 x g (1.000 rpm) and stop the centrifugation). Remove the cover and place the DNA Binding Plate B on the top of **another 2 ml Collection Plate**.
   Transfer the suspension completely into each well of the DNA Binding Plate B. Cover the DNA Binding Plate B with the Plate Lid.
   Load the whole block (DNA Binding Plate B/ 2 ml Collection Plate) into the holder and place the whole assembly in the rotor bucket.
   Centrifuge at 1.400 - 1.700 x g (3.700 – 4.000 rpm) for 6 min at RT. Take the DNA Binding Plate B/ 2 ml Collection Plate out of the centrifuge. Remove the Plate Lid and discard the filtrate. Place the DNA Binding Plate B back to the top of the 2 ml Collection Plate.

6. Add 600 µl **Wash Buffer I** to each well of the DNA Binding Plate B. Cover the DNA Binding Plate B with the Plate Lid. Load the whole block (DNA Binding Plate B/2 ml Collection Plate) into the holder and place the whole assembly in the rotor bucket. Centrifuge at 1.400 - 1.700 x g (3.700 – 4.000 rpm) for 3 min at RT. Remove the cover

and discard the filtrate and place the DNA Binding Plate B back to the top of the 2 ml Collection Plate.

7. Add 700 µl Wash Buffer II to each well of the DNA Binding Plate B. Cover the DNA Binding Plate B with the Plate Lid. Load the whole block (DNA Binding Plate B/2 ml Collection Plate) into the holder and place the whole assembly in the rotor bucket. Centrifuge at 1.400 - 1.700 x g (3.700 – 4.000 rpm) for 3 min at RT. Remove the cover and empty the 2 ml Collection Plate and dry its upper side with paper. Place the DNA Binding Plate B onto a clean surface (paper towel).

Repeat step 7

8. Cover the DNA Binding Plate B with the Plate Lid and put it on top of the 2 ml Collection Plate. Load the whole block (DNA Binding Plate B/2 ml Collection Plate) into the holder and place the whole assembly in the rotor bucket of the centrifuge. Centrifuge at maximum speed for at least 10 min at RT to eliminate any traces of ethanol. Take the DNA Binding Plate B/ 2 ml Collection Plate out of the centrifuge, remove the cover and place the plate on a clean paper towel. Discard the 2 ml Collection Plate.

9. **Place the DNA Binding Plate B on top of a Elution Plate L.**
Add 100 µl **Elution Buffer** prewarmed to 65°C directly onto the membrane in each well and incubate for 5 min at ambient temperature. Cover the DNA Binding Plate B with the Plate Lid and place the whole block (DNA Binding Plate B/ Elution Plate L) in the rotor bucket of the centrifuge. Centrifuge for 3 min at 1.400 - 1.700 x g (3.700 – 4.000 rpm). Take the DNA Binding Plate B and the Elution Plate L out of the centrifuge very carefully in order to avoid cross-contaminations with adherent fluid. Discard the DNA Binding Plate B. Seal the Elution Plate L with a Sealing Foil for a sure storage of the DNA-samples.

Protocol 2: Purification of bacterial DNA from bacterial cultures using a centrifuge

Please read the protocols prior to the start of the preparation and complete preparing steps

Attention: *Please be aware, that you have to prepare the Binding Buffer HL – see page 16*

Sample Preparation
For isolation of DNA from bacteria pellets (max. 1 x 10^9 bacteria cells) take an aliquot of the bacteria culture and spin down at 9.300 x g (10.000 rpm) for 3 min.
Remove carefully the complete supernatant.

Normalization:
Add 100 µl water or 1x PBS to each pellet and resuspend the pellet by pipetting up and down. Transfer the resuspended sample into one well of the **2 ml Collection Plate** and proceed the purification process.

Follow the Protocol 1 from step 2

Protocol 3: Purification of bacterial DNA from food samples using a centrifuge

Please read the protocols prior to the start of the preparation and complete preparing steps

Attention: *Please be aware, that you have to prepare the Binding Buffer HL – see page 16*

Sample Preparation
§ 35 LMBG (Lebensmittel- und Bedarfsgegenständegesetzes)

Take 25 g of the food material and homogenize the material.
Add to the homogenized material 225 ml of the recommended culture media (e.g. Fraser media) and cultivate the recommended time (24h).
Take a 1 ml aliquot of this culture and spin down at 9.300 x g (10.000 rpm) for 3 min.
Remove careful completely the supernatant.

Normalization:
Add 100 µl water or 1x PBS to each pellet and resuspend the pellet by pipetting up and down. Transfer the resuspended sample into one well of the **2 ml Collection Plate** and proceed the purification process.

Follow the Protocol 1 from step 2

Protocol 4: Purification of bacterial DNA from swab samples using a centrifuge

Please read the protocols prior to the start of the preparation and complete preparing steps

Attention: *Please be aware, that you have to prepare the Binding Buffer HL – see page 16*

Sample Preparation
It is recommended to rinse each swab with 500 µl 1x PBS or water and use an 100 µl aliquot of the rinsed water for the extraction of the bacterial DNA. If the swab will be delivered in a stabilization media, use 100 µl of this medium.

Transfer 100 µl of the rinsed liquid or of the transportation media of one swab into the well of the **2 ml Collection Plate** and proceed the purification process.

Follow the Protocol 1 from step 2

Protocol 5: Purification of bacterial DNA from urine samples using a centrifuge

Please read the protocols prior to the start of the preparation and complete preparing steps

Attention: *Please be aware, that you have to prepare the Binding Buffer HL – see page 16*

Sample Preparation

Centrifuge the collected urine sample (15 – 50 ml) for 15 minutes at 1.500 x g (3500 rpm).
Decant the supernatant carefully and resuspend the sediment with 3 ml 1 x PBS.
Centrifuge for 5 minutes at 1.500 x g (3500 rpm).
Decant the supernatant carefully but completely by inverting the tube for some minutes.

It is important to remove the supernatant completely ! Residual amounts of liquid will have negative influence on the further extraction procedure!

Normalization:
Add 100 µl water or 1x PBS to each pellet and resuspend the pellet by pipetting up and down. Transfer the resuspended sample into one well of the **2 ml Collection Plate** and proceed the purification process.

Follow the Protocol 1 from step 2

Protocol 6: Purification of bacterial DNA from small biopsy samples using a centrifuge

Please read the protocols prior to the start of the preparation and complete preparing steps

Attention: *Please be aware, that you have to prepare the Binding Buffer HL – see page 16*

Sample Preparation

Homogenize the 5 –10 mg biopsy samples in a Mixer Mill or under liquid nitrogen with mortar and pestle .

Normalization:
Add 100µl water or 1x PBS to each homogenate and resuspend the homogenate by pipetting up and down. Transfer the resuspended sample into one well of the **2 ml Collection Plate** and proceed the purification process.

Follow the Protocol 1 from step 2

The lysis step with Proteinase K may be extended to 15 min
The lysis step with Lysis Buffer HL, may be extended to 10 min

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Troubleshooting

Problem	Probable cause	Comments and suggestions
clogged DNA Binding Plate B	viscous lysates, too much starting material	reduce amount of starting material or dilute the lysate in **Lysis Buffer HL** 1:1 and use the half before adding **Binding Buffer HL** if this happens during the run pierce well with a needle increase incubation time with **Lysis Buffer HL**
low amounts of extracted DNA	insufficient lysis	increase time for lysis with **Lysis Buffer HL** reduce amount of starting material mix the sample vigorously with **Lysis Buffer HL**, before and during lysis (by pipetting up and down or shaking) incubate at 65°C after adding **Lysis Buffer HL** in a incubator, cool down and place plate in same orientation back. continuously shaking of the lysates during lysis will improve lysis efficiency homogenizing or sample crushing will improve lysis efficiency
	insufficient binding of DNA onto the membrane	use proper volume of **Lysis Buffer HL** and Binding Buffer HL mix sample thoroughly with **Binding Buffer HL** by pipetting up and down prior to transfer the sample onto the DNA Binding Plate B make sure that the correct amount of ethanol is added to the **Wash Buffers** and stored correctly
	incomplete elution	increase centrifugation time in step 15 increase incubation time with pre-warmed Elution Buffer to 10 min pre-warm Elution Buffer up to 80°C use higher volume of **Elution Buffer**
	low DNA concentration	eluting DNA with lower amount of **Elution Buffer**
eluted DNA is colored	insufficient washing	wash again with **Wash Buffer II**
problems with subsequent applications (e.g. with PCR)	eluat contains ethanol	verify centrifugation time and speed (step 15).If necessary increase centrifugation time to remove ethanol.
	eluat contains salt	**Wash Buffer** should have room temperature. **Wash Buffer** shouldn't contain any precipitates, if there are any precipitates solve them by careful warming up to room temperature
	too much/ too low template DNA was used	optimized needed amount of template DNA
	DNA is contaminated with RNA	perform RNAse digestion

Reference:
Sambrock, J. , Fritsch, E.F. and Maniatis T. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Appendix

General notes on handling DNA

1) Nature of DNA

The length and physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR.

2) Storage of DNA

A working stock of DNA can be stored at 2 – 4˚C for several weeks. For long term storage DNA should be stored at -20˚C, but storing at – 20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

3) Drying, dissolving and pipetting DNA

Avoid overdrying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

4) DNA Yield

The amount of purified DNA from the whole blood depends on the leucocytes content, sample source, transport, storage and age. Various different primary tubes and anticoagulants (except heparin) can be used to collect blood samples for the **Invisorb®** procedure.

Ordering information

Product	Catalogue No.	Package Size
Invisorb® Universal Bacteria HTS 96 Kit/ C	7033300200	2 x 96 preps
Invisorb® Universal Bacteria HTS 96 Kit/ C	7033300300	4 x 96 preps
Invisorb® Universal Bacteria HTS 96 Kit/ C	7033300400	24 x 96 preps
InviMag® Universal Bacteria Kit/ KF96	7433300100	1 x 96 preps
InviMag® Universal Bacteria Kit/ KF96	7433300100	5 x 96 preps
InviMag® DNA Bacteria Mini Kit/ KFmL	2433150100	15 preps
InviMag® DNA Bacteria Mini Kit/ KFmL	2433150200	75 preps
RTP® Bacteria DNA Mini Kit	1033200200	50 preps
RTP® Bacteria DNA Mini Kit	1033200300	250 preps
RTP® Mycobacteria Kit	1033220200	50 preps
RTP® Mycobacteria Kit	1033220300	250 preps
Lysozyme	3020401300	150 mg
Lysozyme Buffer	3020401400	15 ml

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